OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® BAC/PAC Maxi Kit

D2154-00	2 preps
D2154-01	5 preps

April 2013

For research use only.Not intended for diagnostic testing.

E.Z.N.A.® BAC/PAC Maxi Kit

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Introduction and Overview

The E.Z.N.A.® family of products is an innovative system that radically simplifies extraction and purification of nucleic acids from a variety of sources. Key to the system is Omega Bio-tek's proprietary HiBind® matrix that avidly, but reversibly, binds DNA under certain optimal conditions, allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or low salt buffer.

The E.Z.N.A.® BAC/PAC DNA Maxi Kit combines the power of HiBind® technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high-quality BAC/PAC DNA. Omega Bio-tek's HiBind® BAC/PAC DNA Maxi Columns facilitate the binding, washing, and elution steps, thus enabling multiple samples to be simultaneously processed.

Yields vary according to copy number, *E. coli* strain, and growth conditions. Typically, 200 mLovernight culture in an 2x YT medium typically produces 20-50 µg BAC DNA. Isolated BAC/PAC DNA is suitable for automated fluorescent DNA sequencing, restriction enzyme digestion, and other manipulations.

New in this Edition:

- This manual has been edited for content and redesigned to enhance user readability.
- Equilibration Buffer is no longer included with this kit. An optional Column Equilibration Protocol has been added to the protocol for your convenience.
- Equilibration Buffer is replaced with 3M NaOH provided by the user.
- HB Buffer has been replaced with HBC Buffer. HBC Buffer must be diluted with isopropanol (provided by user) before use.
- HB Buffer is no longer included with this kit.

Kit Contents

Product	D2154-00	D2154-01
Purifications	2	5
HiBind® BAC/PAC DNA Maxi Columns	2	5
50 mL Collection Tubes	2	5
T1 Buffer	40 mL	90 mL
T2 Buffer	40 mL	90 mL
T3 Buffer	40 mL	90 mL
BAC Binding Buffer	15 mL	30 mL
HBC Buffer	18 mL	40 mL
SPM Wash Buffer	18 mL	42 mL
RNase A	100 µL	300 µL
Elution Buffer*	10 mL	30 mL
User Manual	✓	✓

* Elution Buffer is 10 mM Tris HCl, pH 8.5

Storage and Stability

All of the E.Z.N.A.® BAC/PAC Maxi DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. RNase A should be stored at 2-8˚C. T1 Buffer (once RNase A is added) should be stored at 2-8˚C. All remaining components should be stored at room temperature.

Preparing Reagents

1. Dilute BAC Binding Buffer with isopropanol as follows and store at room temperature.

Kit	Isopropanol to be Added
D2154-00	45 mL
D2154-01	90 mL

2. Dilute SPM Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D2154-00	42 mL
D2154-01	98 mL

3. Dilute HBC Buffer with isopropanol as follows and store at room temperature.

Kit	Isopropanol to be Added
D2154-00	7 mL
D2154-01	16 mL

4. Add the vial of RNase A to the bottle of T1 Buffer and store at 2-8 °C.

Guidelines for Vacuum Manifold

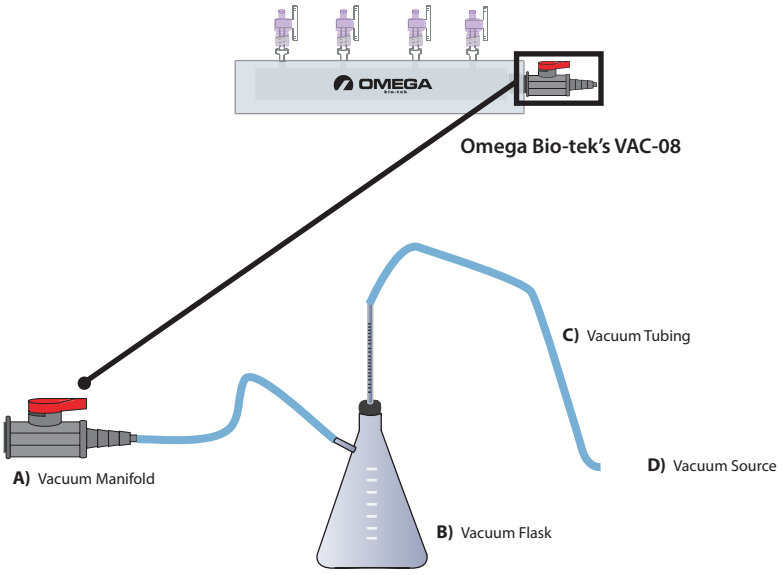
The following is required for use with the Vacuum/Spin Protocol:

A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-08)
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma AldrichVM20, Promega Vacman®, or manifold with standard Luer connector
B) Vacuum Flask
C) Vacuum Tubing
D) Vacuum Source (review tables below for pressure settings)

Manifold	Recommended Pressure (mbar)
VAC-08	-200 to -600

Conversion from millibars:	Multiply by:
Millimeters of mercury (mmHg)	0.75
Kilopascals (kPa)	0.1
Inches of mercury (inch Hg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Vacuum Setup:



E.Z.N.A.® BAC/PAC Kit - Vacuum Protocol

Materials and Equipment to be Supplied by User:

- Refrigerated centrifuge with swing-bucket rotor capable of at least 3,000 x *g* and capable of 4°C
- Vortexer
- Vacuum manifold
- Nuclease-free 50 mL centrifuge tubes (Falcon® tubes recommended)
- Ice bucket
- Isopropanol
- 100% ethanol
- Optional: Incubator capable of 65°C
- Optional: Sterile deionized water or TE Buffer
- Optional: 3M NaOH

Before Starting:

- Prepare T1 Buffer, BAC Binding Buffer, HBC Buffer, and SPM Wash Buffer according to the Preparing Reagents section on Page 4
- Prepare an ice bucket
- Chill T3 Buffer on ice
- Optional: Set an incubator, heat block, or water bath to 65°C
- Optional: Heat Elution Buffer to 65°C

1. Isolate a single colony from a freshly streaked selective plate and inoculate a culture of 100-200 mL 2xYT medium containing the appropriate antibiotic. Incubate for ~16-20 hours at 37°C with vigorous shaking (~300 rpm).

   Note: Optimal growth conditions are vital in obtaining maximal BAC DNA yields. To achieve the best conditions, use a single colony from a freshly transformed or freshly streaked plate to inoculate a 2-5 mL starter culture containing the appropriate antibiotic. Incubate for ~8 hours at 37°C with vigorous shaking (~300 rpm). Use the starter culture to inoculate an appropriate volume of warm growth media containing the desired antibiotic. Grow at 37°C for 16-20 hours with vigorous shaking(~300 rpm). Use a flask or vessel with a volume of at least 3-4 times the volume of the culture and dilute the starter culture 1/500 to 1/1000 into growth media. If you are using a frozen glycerol stock as the inoculum, streak it onto an agar plate containing the appropriate antibiotic. Inoculate a single colony into a 2-5 mL starter culture as described above.

2. Centrifuge 100-200 mL culture at 3,500-5,000 x *g* for 10-15 minutes at room temperature.

3. Decant or aspirate and discard the culture media.

   Note: To ensure that all traces of the medium are removed, use a clean paper towel to blot excess liquid from the wall of the vessel.

4. Add 16 mL T1 Buffer/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.

   Note: RNase A must be added to T1 Buffer before use. Please see the instructions in the Preparing Reagents section on Page 4.

5. Add 16 mL T2 Buffer. Invert and gently rotate the tube 10-20 times to obtain a clear lysate. A 2-3 minute incubation may be necessary.

   Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower BAC purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store T2 Buffer tightly capped when not in use to avoid acidification from CO_2 in the air.

6. Add 16 mL cold T3 Buffer. Immediately invert several times until a flocculent white precipitate forms.

   Note: It is vital that the solution is mixed thoroughly and immediately after the addition of T3 Buffer to avoid localized precipitation. If the mixture still appears viscous, brownish, or conglobated, more mixing is required to completely neutralize the solution.

7. Incubate on ice for 10 minutes.

8. Centrifuge at 3,000-5,000 x *g* for 15 minutes at 4°C .

   Note: For faster removal of the precipitated bacterial cell material, one may order Omega's Lysate Clearance Filter Syringes to replace centrifugation step. This filter cartridge completely removes SDS precipitates and clears bacterial lysates using a filter syringe.

9. Transfer the cleared supernatant by CAREFULLY aspirating it into an appropriate vessel. Be careful not to disturb the pellet and that no cellular debris is transferred to the new tube.

   Note: When transferring the supernatant, precipitates may float to the top of the supernatant. Carefully insert the pipettor around the floating precipitates and transfer the cleared supernatant only.

10. Add 20 mL BAC Binding Buffer. Invert the tube 20 times to mix thoroughly.

    Note: BAC Binding Buffer must be diluted with isopropanol prior to use. Please see Page 4 for instructions.

11. Let sit at room temperature for 2-5 minutes.

12. Prepare the vacuum manifold according to manufacturer's instructions.

13. Connect the HiBind® BAC/PAC DNA Maxi Column to the vacuum manifold.

Optional Protocol for Column Equilibration

1. Add 3 mL 3M NaOH to the HiBind® BAC/PAC DNA Maxi Column.
2. Let sit for 4 minutes at room temperature.
3. Turn on the vacuum to draw the NaOH through the column.
4. Turn off the vacuum.

14. Transfer 20 mL cleared supernatant from Step 11 by CAREFULLY aspirating it into the HiBind® BAC/PAC DNA Maxi Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® BAC/PAC DNA Maxi Column.

15. Turn on the vacuum source to draw the sample through the column.

16. Turn off the vacuum.

17. Repeat Steps 14-16 until all of the cleared supernatant has been transferred to the column.

18. Add 10 mL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol prior to use. Please see Page 4 for instructions.

19. Turn on the vacuum source to draw the HBC Buffer through the column.

20. Turn off the vacuum.

21. Add 20 mL SPM Wash Buffer.

    Note: SPM Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

22. Turn on the vacuum source to draw the SPM Wash Buffer through the column.

23. Continue to apply the vacuum for 5 minutes after the SPM Wash Buffer has passed through the column.

24. Transfer the HiBind® BAC/PAC DNA Maxi Column to a 50 mL Collection Tube.

25. Centrifuge the empty HiBind® BAC/PAC DNA Maxi Column for 10 minutes at maximum speed to dry the column matrix.

    Note: It is important to dry the HiBind® BAC/PAC DNA Maxi Column matrix before elution. Residual ethanol may interfere with downstream applications.

Note: For maximal yield and higher concentration of BAC DNA, see the alterative elution protocol on Page 11. For the standard elution protocol, proceed with the protocol below.

Optional: To ensure the removal of residual ethanol from the column, choose one of the following methods below to further dry the column before proceeding with DNA elution.

A. Place the column into a vacuum container to dry for 15 minutes.
   1. Transfer the column into a vacuum chamber at room temperature. Any device connected to a vacuum source may be used.
   2. Seal the chamber and apply vacuum for 15 minutes.
   3. Remove the column and proceed to Step 26.

B. Bake the column in a vacuum oven or incubator at 65°C for 10 minutes. Remove the column and proceed to Step 26.

26. Transfer the HiBind® BAC/PAC DNA Maxi Column to a 50 mL centrifuge tube (not provided).

27. Add 0.7-2.0 mL Elution Buffer, sterile deionized water, or TE buffer directly onto the column matrix.

28. Let sit for 5-10 minutes at room temperature.

29. Centrifuge at a maximum speed for 5 minutes.

30. Store DNA at -20°C.

    Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration. The second elution may be performed using the first eluate to maintain a higher DNA concentration. Heating the Elution Buffer to 65°C prior to the elution step may increase yields.

E.Z.N.A.® BAC/PAC Kit - Centrifugation Protocol

Materials and Equipment to be Supplied by User:

- Refrigerated centrifuge with swing-bucket rotor capable of at least 3,000 x *g* and capable of 4°C
- Vortexer
- Vacuum manifold
- Nuclease-free 50 mL centrifuge tubes (Falcon® tubes recommended)
- Ice bucket
- Isopropanol
- 100% ethanol
- Optional: Incubator capable of 65°C
- Optional: Sterile deionized water or TE Buffer
- Optional: 3M NaOH

Before Starting:

- Prepare T1 Buffer, BAC Binding Buffer, HBC Buffer, and SPM Wash Buffer according to the Preparing Reagents section on Page 4
- Prepare an ice bucket
- Chill T3 Buffer on ice
- Optional: Set an incubator, heat block, or water bath to 65°C
- Optional: Heat Elution Buffer to 65°C

1. Isolate a single colony from a freshly streaked selective plate and inoculate a culture of 100-200 mL 2xYT medium containing the appropriate antibiotic. Incubate for ~16-20 hours at 37°C with vigorous shaking (~300 rpm).

   Note: Optimal growth conditions are vital in obtaining maximal BAC DNA yields. To achieve the best conditions, use a single colony from a freshly transformed or freshly streaked plate to inoculate a 2-5 mL starter culture containing the appropriate antibiotic. Incubate for ~8 hours at 37°C with vigorous shaking (~300 rpm). Use the starter culture to inoculate an appropriate volume of warm growth media containing the desired antibiotic. Grow at 37°C for 16-20 hours with vigorous shaking(~300 rpm). Use a flask or vessel with a volume of at least 3-4 times the volume of the culture and dilute the starter culture 1/500 to 1/1000 into growth media. If you are using a frozen glycerol stock as the inoculum, streak it onto an agar plate containing the appropriate antibiotic. Inoculate a single colony into a 2-5 mL starter culture as described above.

2. Centrifuge 100-200 mL culture at 3,500-5,000 x *g* for 10-15 minutes at room temperature.

3. Decant or aspirate and discard the culture media.

   Note: To ensure that all traces of the medium are removed, use a clean paper towel to blot excess liquid from the wall of the vessel.

4. Add 16 mL T1 Buffer/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.

   Note: RNase A must be added to T1 Buffer before use. Please see the instructions in the Preparing Reagents section on Page 4.

5. Add 16 mL T2 Buffer. Invert and gently rotate the tube 10-20 times to obtain a clear lysate. A 2-3 minute incubation may be necessary.

   Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower BAC purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store T2 Buffer tightly capped when not in use to avoid acidification from CO_2 in the air.

6. Add 16 mL cold T3 Buffer. Immediately invert several times until a flocculent white precipitate forms.

   Note: It is vital that the solution is mixed thoroughly and immediately after the addition of T3 Buffer to avoid localized precipitation. If the mixture still appears viscous, brownish, or conglobated, more mixing is required to completely neutralize the solution.

7. Incubate on ice for 10 minutes.

8. Centrifuge at 3,000-5,000 x *g* for 15 minutes at 4°C .

   Note: For faster removal of the precipitated bacterial cell material, one may order Omega's Lysate Clearance Filter Syringes to replace centrifugation step. This filter cartridge completely removes SDS precipitates and clears bacterial lysates using a filter syringe.

9. Transfer the cleared supernatant by CAREFULLY aspirating it into an appropriate vessel. Be careful not to disturb the pellet and that no cellular debris is transferred to the new tube.

   Note: When transferring the supernatant, precipitates may float to the top of the supernatant. Carefully insert the pipettor around the floating precipitates and transfer the cleared supernatant only.

10. Add 20 mL BAC Binding Buffer. Invert the tube 20 times to mix thoroughly.

    Note: BAC Binding Buffer must be diluted with isopropanol prior to use. Please see Page 4 for instructions.

11. Let sit at room temperature for 2-5 minutes.

12. Insert a HiBind® BAC/PAC DNA Maxi Column into a 50 mL Collection Tube (provided).

Optional Protocol for Column Equilibration

1. Add 3 mL 3M NaOH to the HiBind® BAC/PAC DNA Maxi Column.
2. Let sit for 4 minutes at room temperature.
3. Centrifuge at 3,000 x *g* for 3 minutes.
4. Discard the filtrate and reuse the Collection Tube.

13. Transfer 20 mL cleared supernatant from Step 11 by CAREFULLY aspirating it into the HiBind® BAC/PAC DNA Maxi Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® BAC/PAC DNA Maxi Column.

14. Centrifuge at 4,000 x *g* for 5 minutes.

15. Discard the filtrate and reuse the Collection Tube.

16. Repeat Steps 13-15 until all of the cleared supernatant has been transferred to the HiBind® BAC/PAC DNA Maxi Column.

17. Add 10 mL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol prior to use. Please see Page 4 for instructions.

18. Centrifuge at 4,000 x *g* for 5 minutes.

19. Discard the filtrate and reuse the Collection Tube.

20. Add 20 mL SPM Wash Buffer.

    Note: SPM Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

21. Centrifuge at 4,000 x *g* for 5 minutes.

22. Discard the filtrate and reuse the Collection Tube.

23. Centrifuge the empty HiBind® BAC/PAC DNA Maxi Column for 10 minutes at maximum speed to dry the column matrix.

    Note: It is important to dry the HiBind® BAC/PAC DNA Maxi Column matrix before elution. Residual ethanol may interfere with downstream applications.

Note: For maximal yield and higher concentration of BAC DNA, see the alterative elution protocol on Page 11. For the standard elution protocol, proceed with the protocol below.

Optional: To ensure the removal of residual ethanol from the column, choose one of the following methods below to further dry the column before proceeding with DNA elution.

A. Place the column into a vacuum container to dry for 15 minutes.
   1. Transfer the column into a vacuum chamber at room temperature. Any device connected to a vacuum source may be used.
   2. Seal the chamber and apply vacuum for 15 minutes.
   3. Remove the column and proceed to Step 26.

B. Bake the column in a vacuum oven or incubator at 65°C for 10 minutes. Remove the column and proceed to Step 26.

24. Transfer the HiBind® BAC/PAC DNA Maxi Column to a 50 mL centrifuge tube (not provided).

25. Add 0.7-2.0 mL Elution Buffer, sterile deionized water, or TE buffer directly onto the column matrix.

26. Let sit for 5-10 minutes at room temperature.

27. Centrifuge at a maximum speed for 5 minutes.

28. Store DNA at -20°C.

    Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration. The second elution may be performed using the first eluate to maintain a higher DNA concentration. Heating the Elution Buffer to 65°C prior to the elution step may increase yields.

E.Z.N.A.® BAC/PAC Kit - Alternative Protocol For BAC Elution

Materials and Equipment to be Supplied by User:

- Refrigerated centrifuge with swing-bucket rotor capable of at least 3,000 x *g* and capable of 4°C
- Vortexer
- Nuclease-free 50 mL centrifuge tubes (Falcon® tubes recommended)
- Nuclease-free 15 mL centrifuge tubes
- 70% ethanol
- 3M sodium acetate
- Ice bucket
- Optional: Incubator capable of 65°C
- Optional: Sterile deionized water or TE Buffer

Before Starting:

- Prepare ice bucket.
- Chill 70% ethanol on ice.
- Optional: Set an incubator, heat block, or water bath to 65°C
- Optional: Heat Elution Buffer to 65°C

1. Transfer the HiBind® BAC/PAC DNA Maxi Column to a clean 50 mL centrifuge tube.

2. Add 3 mL Elution Buffer, sterile deionized water, or TE buffer directly onto the column matrix.

3. Let it sit at room temperature for 5 minutes.

4. Centrifuge at 4,000 x *g* for 5 minutes.

Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration. The second elution may be performed using the first eluate to maintain a higher DNA concentration. Heating the Elution Buffer to 65°C prior to the elution step may increase yields.

5. Carefully transfer the eluate to a nuclease-free 15 mL centrifuge tube.

6. Add 1/10 volume 3M sodium acetate and 7/10 volume isopropanol. Vortex to mix thoroughly.

7. Centrifuge at 5,000 × *g* for 20 minutes at 4°C.

8. Carefully decant and discard the supernatant without disturbing the pellet.

9. Add 5 mL ice-cold 70% ethanol. Vortex to completely resuspend the pellet.

10. Centrifuge at 3,000 × *g* for 10 minutes.

11. Carefully decant and discard the supernatant without disturbing the pellet.

12. Let sit for 5-10 minutes to air dry.

13. Add 200-500 µL TE Buffer or sterile deionized water.

14. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Low DNA yields	
Poor cell lysis	• Only use LB or YT medium containing ampicillin. Do not use more than 200 mL with high copy BACs. • Cells may not be dispersed adequately prior to addition of T2 Buffer. Vortex cell suspension to completely disperse. • Increase incubation time with T2 Buffer to obtain a clear lysate.
Bacterial culture overgrown or not fresh	Do not incubate cultures for more than 16 hours at 37°C. Storage of cultures for extended periods prior to BAC DNA isolation is detrimental.
No DNA eluted	
SPM Wash Buffer not diluted with ethanol	Prepare SPM Wash Buffer according to the Preparing Reagents section on Page 4.
HBC Buffer not diluted with isopropanol	Prepare HBC Buffer according to the Preparing Reagents section on Page 4.
High molecular weight DNA contamination of product	
Over mixing of cell lysate upon addition of T2 Buffer	Do not vortex or mix aggressively after adding T2 Buffer. Adequate mixing is obtained by simply inverting and rotating tube to cover walls with viscous lysate.
Optical densities do not agree with DNA yield on agarose gel	
Trace contaminants eluted from column increase A_{260}	Make sure to wash column as instructed. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantitation.
RNA visible on agarose gel	
RNase A not added to T1 Buffer	Add 1 vial of RNase to each bottle of T1 Buffer.

BAC DNA floats out of well while loading agarose gel	
Ethanol not completely removed from column following wash steps	Centrifuge column as instructed to dry.
BAC DNA will not perform in downstream application	
Traces of ethanol remain on column prior to elution	The column must be washed with ethanol and dried before elution. Ethanol precipitation may be required following elution.
A_{260}/A_{280} ratio is high or low	
SPM Wash Buffer was diluted with ethanol containing impurities	Check the absorbency of the ethanol between 230-280 nm. Do not use ethanol with high absorbency.
Purified BAC has RNA contamination	Ensure that RNase A was added to T1 Buffer before use.
Background reading is high due to the silica fines	Centrifuge the purified DNA for 1-2 minutes, transfer the sample to a new tube, and measure the OD again.

Notes:

HiBind®, E.Z.N.A.®, and MicroElute® are registered trademarks of Omega Bio-tek, Inc.
Qiagen®, QIAvac® and Vacman® are all trademarks of their respective companies.
PCR is a patented process of Hoffman-La Roche. Use of the PCR process requires a license.